Correction and Apology

The original version of the following paper was published in the *European Journal of Cancer*, Vol. 31A, No. 6, pp. 924–928, 1995. Unfortunately, Figure 5 was incorrectly labelled. The paper is therefore being withdrawn and the correct version is republished in full below.

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Clinical Significance of Serum S100 in Metastatic Malignant Melanoma

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The clinical significance of serum S100 was assessed in comparison to neuron-specific enolase (NSE) in 126 patients with malignant melanoma: 80 patients with clinical stage I/II, 23 patients with stage III and 23 patients with stage IV according to the criteria of the American Joint Committee on Cancer (AJCC). Using cut-off values of 0.15 μg/l for S100 and 12.5 μg/l for NSE, the sensitivity was found to be 1.3% (1/80) for S100 and 8.75% (7/80) for NSE in patients with stage I/II, 8.7% (2/23) for S100 and 13% (8/23) for NSE in patients with stage III, and 73.9% (17/23) for S100 and 34.8% (8/23) for NSE in patients with stage IV disease ($P < 0.05$). In 6 patients with stage III/IV tumours, serial measurement of serum S100 and NSE was performed. A rise of serum S100 indicated progression of the disease; a decline indicated response to treatment. Our preliminary results support the value of serum S100 as an adjunct to the clinical staging and monitoring of metastatic malignant melanoma.

Key words: serum S100 protein, neuron-specific enolase, malignant melanoma, tumour marker
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INTRODUCTION

THE INCIDENCE of melanoma has increased in the past 40 years [1, 2]. Melonoma can metastasise to any organ, therefore, follow-up after surgery is difficult. Patients with metastatic malignant melanoma are generally incurable. However, new therapeutic strategies, for example with cytokines, are being developed [3], and a major challenge facing clinical biochemical research is to detect specific and reliable serum markers that are of value in diagnosing and monitoring progression of the disease. Recently, it was demonstrated that neuron-specific enolase (NSE) is a useful prognostic factor for metastatic malignant melanoma [4, 5].

In previous studies, protein S100 was found in malignant melanoma tissue, but was absent from tissue samples of non-melanotic tumours, from normal skin and normal lymph nodes [6]. In one study, it was observed that protein S100 was elevated in serum of patients with metastatic malignant melanoma [7].

S100 is an acidic calcium-binding protein with a molecular weight of 21 000 found in the nervous system of vertebrates [8]. It is a dimerous protein, consisting of two isomerous subunits (α, molecular weight (MW) 10400; β, MW 10500), whereby each of the possible combinations occurs [9]. Its name derives from its solubility in 100% saturated ammonium sulphate at neutral pH [10]. S100 is located mainly in astrocytes, Schwann cells and satellite cells in sympathetic ganglia [11, 12]. Previous studies suggest that S100 in cerebrospinal fluid and serum could be a useful marker for damage to the nervous system [13, 14].

The aims of this study were (a) to analyse the clinical significance of serum S100 at the time of diagnosis of malignant melanoma, (b) to evaluate the use of serum S100 in the follow-up of patients treated for malignant melanoma, and (c) to compare serum levels of S100 with NSE in patients with malignant melanoma.

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PATIENTS AND METHODS

Serum samples were taken from 126 patients with malignant melanoma admitted to the University Hospital of Bonn from May 1993 to April 1994. In all patients, the diagnosis was proven by histology. Table 1 summarises age and sex of the patients, as well as growth pattern and stage of disease. Staging was performed according to the criteria of the American Joint Committee on Cancer (AJCC)/Union Internationale Contre le Cancer (UICC) based on the tumour–node–metastasis (TNM) system: stage I (T1/T2, N0, M0), stage II (T3/T4, N0, M0), stage III (any T, N1/2, M0) and stage IV (any T, any N, M1) [2]. In patients with stages I and II, blood samples were taken before treatment. Additionally, in 6 patients with stage III/IV, blood samples were taken serially during the course of treatment. The therapies employed were surgery, chemotherapy and immunochemotherapy. The responses were assessed by clinical examination, routine laboratory tests, chest roentgenography, computed tomography (CT), abdominal ultrasound, magnetic resonance imaging (MRI), and bone scan.

The reference groups consisted of 25 healthy people [14 women and 11 men, age 52.6 ± 14.5 years (mean ± S.D.)], and 45 patients with benign skin lesions [25 women and 20 men, age 48.2 ± 18.4 years (mean ± S.D.): 6 patients with neurodermitis, 7 patients with benign acquired naevi, 2 patients with psoriasis, 12 persons with multiple freckling, 8 patients with large congenital naevi and 10 patients after ultraviolet exposure]. All sera were stored at −20°C until assayed and determined blind of clinical information.

Serum S100 was measured by an immmunoradiometric assay (IRMA, Byk-Sangtec Diagnostics, Dietzenbach, Germany). This assay is based on the two-site sandwich method using two different monoclonal antibodies. In the first step of the assay, the samples are incubated with a plastic bead coated with a monoclonal antibody to S100. After washing, a I^{125}-labelled monoclonal antibody is added and incubated. After further washing, the radioactivity bound to the bead is measured in a gamma counter. S100 concentration is calculated using standards with known concentrations of S100 and expressed in μg/l.

The detection limit of the S100 IRMA was 0.15 μ/1. A dilution of a serum sample with a high S100 concentration showed a linear relation between the dilution and S100 concentration ($r = 0.99953$, $P < 0.000001$). Intra-assay coefficients of variation were 3.2% for low values ($n = 10$, mean = 2.8 μg/l) and 3.4% for high values ($n = 10$, mean = 28.2 μg/l). The corresponding interassay coefficients of variation were 10.3% ($n = 10$, mean = 2.9 μg/l) and 6.5% ($n = 10$, mean = 27.6 μg/l), respectively.

Serum NSE was determined using a commercially available radioimmunassay (Pharmacia, Uppsala, Sweden). The detection limit was 2 μg/l. Intra-assay coefficients of variation were 7.3% for low values ($n = 10$, mean = 8.2 μg/l) and 3.2% for high values ($n = 10$, mean = 34.8 μg/l). The corresponding interassay coefficients of variation were 7.1% ($n = 10$, mean = 8.4 μg/l) and 8% ($n = 10$, mean = 33.5 μg/l), respectively. Values exceeding 12.5 μg/l were considered as elevated.

Statistical analysis was performed using the χ^2 test and Spearman's rank correlation. A value of $P < 0.05$ was considered to be significant.

Table 1. Sex, age, stage of the disease and growth pattern of the primary tumour in 126 patients with malignant melanoma

	Stage I/II	Stage III	Stage IV
n	80	23	23
Females/males	33/47	10/13	11/12
Age (mean ± S.D.)	54.7 ± 16.6	65.7 ± 9.9	54.2 ± 13.7
Growth pattern (*n*)			
SSM	47	7	5
ALM	4	7	3
NM	19	7	3
LMM	2	0	0
HMV	8	2	9
CM	0	0	3

SSM, superficial spreading melanoma; ALM, acral lentiginous melanoma; NM, nodular melanoma; LMM, lentigo malignant melanoma; HMV, histological melanoma variants; CM, choroid melanoma.

RESULTS

In the reference group, consisting of 45 patients with benign skin lesions and 25 healthy people, serum S100 concentrations were < 0.15 μg/l in all cases. S100 was detectable only in 1 of 80 patients without metastases (stage I/II). Values exceeding 0.15 μg/l were found in 2 of the 23 patients with stage III (sensitivity 8.7%) and 17 of the 23 patients with stage IV (sensitivity 73.9%). The sensitivity for metastatic malignant melanoma stage III/IV was 41.3% (19/46 patients). Overall, for malignant melanoma (all stages), the diagnostic sensitivity of serum S100 was 15.9%. The data are illustrated in Figure. 1.

In the reference group, NSE concentrations were below the cut-off value in all cases. In 7 of 80 patients without metastases (stage I/II), serum NSE was elevated (sensitivity 8.75%). Increased serum concentrations of NSE (>12.5 μg/l) were found in 3 of 23 patients with stage III (sensitivity 13.0%) and in 8 of 23 patients with stage IV (sensitivity 34.8%). The sensitivity for metastatic malignant melanoma (stage III/IV) was 23.9% (11/46) (Figure 2). Overall, serum NSE showed a sensitivity of 14.3% (18/126) for malignant melanoma (all stages).

The sensitivity of S100 for metastatic malignant melanoma stage IV was significantly higher than that of NSE ($P < 0.05$). The specificity of serum S100 and NSE for malignant melanoma stage III/IV versus stage I/II was 98.8 and 91.25%, respectively.

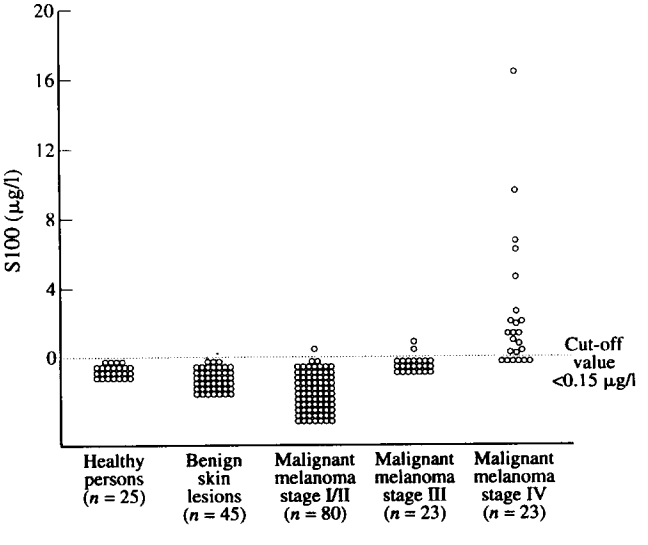


Figure 1. Serum S100 concentrations in healthy people, patients with benign skin lesions and patients with malignant melanoma stage I/II, stage III and stage IV.

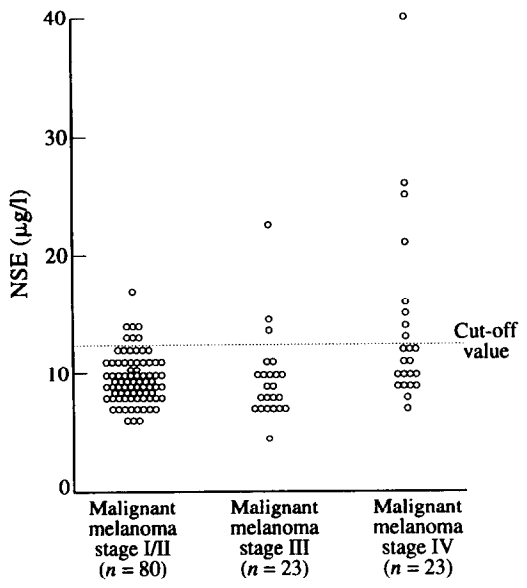


Figure 2. Serum neuron-specific enolase (NSE) concentrations in patients with malignant melanoma stage I/II, stage III and stage IV.

Serum S100 concentrations and serum NSE concentrations in patients with clinical stage III/IV were significantly correlated ($r_s = 0.55; P < 0.01$).

Serum S100 and NSE concentrations were serially measured in 6 patients with malignant melanoma stage III/IV in the course of treatment. All of these patients had initially detectable serum S100 concentrations, whereas only 3 of 6 initially had elevated NSE concentrations. In 2 patients, serum S100 showed a gradual rise accompanying the progression of the disease under treatment. In a stage IV 55-year-old man (lung and bone metastases) (Figure 3), NSE was only slightly elevated and levels did not increase during treatment, until just before death, when there was a rise to 50.6 μg/l. Serum S100 concentrations, however, increased steadily to 321 μg/l. This reflected the progression of the tumour in spite of cytotoxic chemotherapy (carmustine, hydroxycarbamide, dacarbazine). Progression was confirmed by enlargement of lumbar and thoracic bone metastases in MRI scan and enlargement of lung metastases in CT scan. In another 57-year-old man, liver, spleen and bone metastases were already present at the time of diagnosis. Serum S100 and NSE levels were slightly elevated. With progression of liver and spleen metastases, serum S100 and NSE levels began to increase. For example, a representative liver metastasis increased from a diameter of 3 cm to a diameter of 6 cm. When bone and CT scans indicated even further progression, a chemoperfusion of the liver with α-interferon and interleukin-2 was performed. Serum NSE levels remained between 117.3 and 97.7 μg/l, but S100 persistently rose from 7.4 to 98.4 μg/l, corresponding to an enlargement of the liver metastasis to 8 cm and to clinical deterioration until death.

Declining serum S100 concentrations were observed in 4 patients, corresponding to response to therapy, whereas they increased again with progression of the disease (detected by radiological diagnostics). In a 28-year-old woman (Figure 4), metastatic melanoma was diagnosed by histology of retroperitoneal lymph nodes. At this time, serum S100 concentrations were elevated, while serum NSE concentrations were not. Two metastases in the lumbar vertebral column were determined by bone scan. The marked increases of serum S100 and NSE levels correlated with the patient's poor clinical status and the diagnosis of large retroperitoneal tumour masses by CT scan (Figure 5a). Lactate dehydrogenase levels were 681 U/l. A first course of cytotoxic therapy (bleomycin, vincristine, lomustine, dacarbazine) was followed by a good clinical response and a decrease of serum S100 and NSE levels. Lactate dehydrogenase levels decreased to 256 U/l, a CT scan showed a marked decrease of the retroperitoneal tumour masses (Figure 5b) and the analgesic therapy could be discontinued. In spite of two additional courses of chemotherapy, the patient exhibited progressive disease, and serum S100 and NSE levels increased again, until the patient died. Lactate dehydrogenase levels had increased to 856 U/l and later on to 1075 U/l. A CT scan determined an increase of retroperitoneal metastases (Figure 5c), and new metastases in the left abdominal wall and the left groin. On autopsy, metastases were found in the whole body.

When liver metastases were detected in a 65-year-old woman with a choroid melanoma (Figure 6) by a CT scan, serum S100 concentrations were elevated. She was treated several times by chemoembolisation of liver metastases. Serum S100 concentrations markedly decreased thereafter. CT scan showed necrotic areas and a decrease of the size of the metastases. One representa-

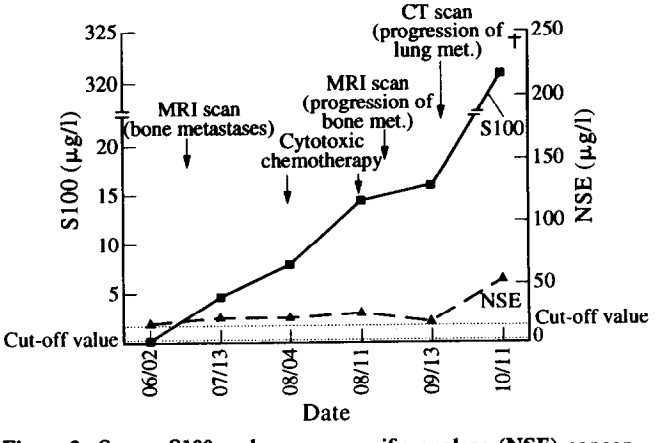


Figure 3. Serum S100 and neuron-specific enolase (NSE) concentrations in a 55-year-old male patient. Primary site of melanoma in the back (superficial spreading melanoma), stage IV (lung and bone metastases, met.). †Time of death.

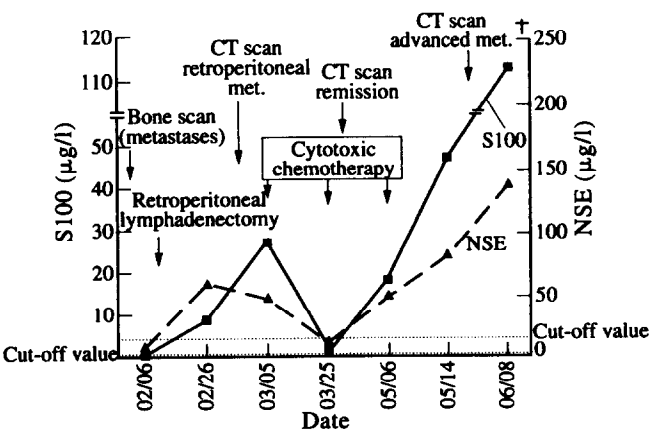
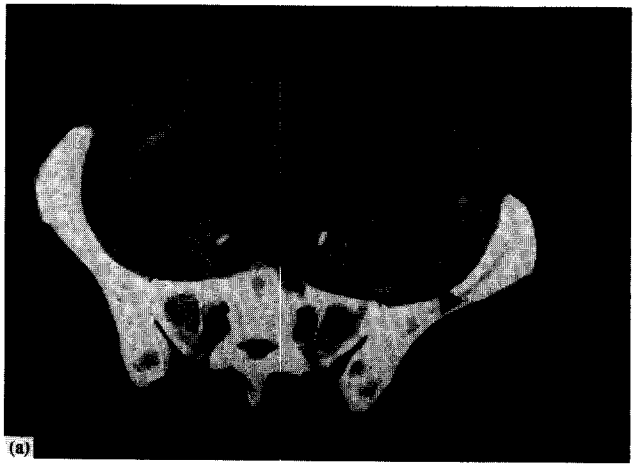
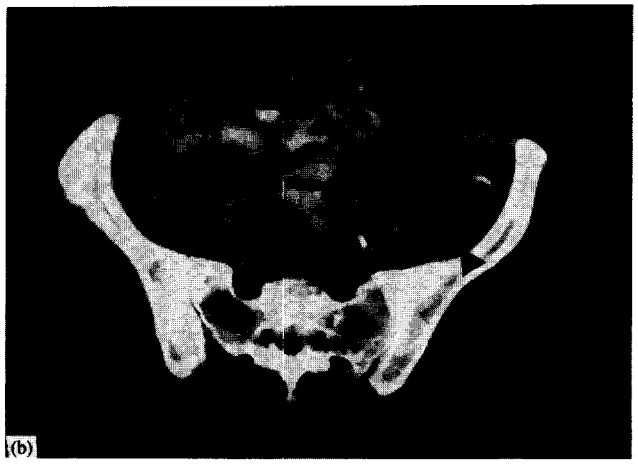


Figure 4. Serum S100 and NSE concentrations in a 28-year-old female patient. Primary site of melanoma unknown, stage IV (liver and lung metastases, met.). †Time of death.





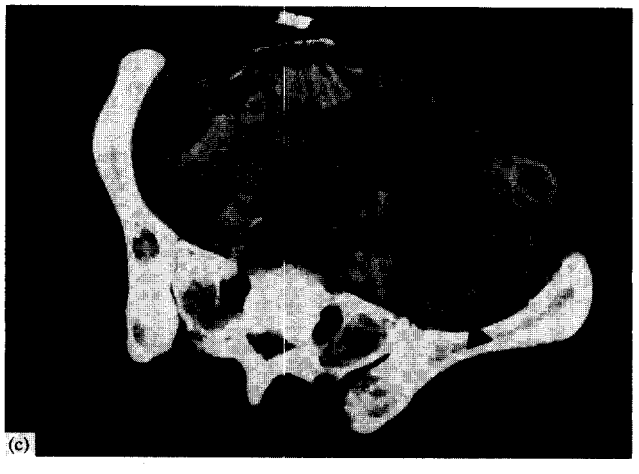


Figure 5. Computed tomography scans of the patient shown in Figure 4. Arrows indicate (a) large retroperitoneal tumour masses before chemotherapy (date 03/02); (b) a decrease of metastases during chemotherapy (date 04/09) and (c) progression of metastases before patient's death (date 06/03).

tive metastasis decreased from a diameter of 5 cm to 3 cm. However, serum S100 concentrations rose again before a CT scan confirmed progression of metastases. Lactate dehydrogenase levels increased to 259 U/l and alkaline phosphatase levels to 1050 U/l, which had been constantly in the upper normal range in the preceeding 6 months. It is remarkable that NSE

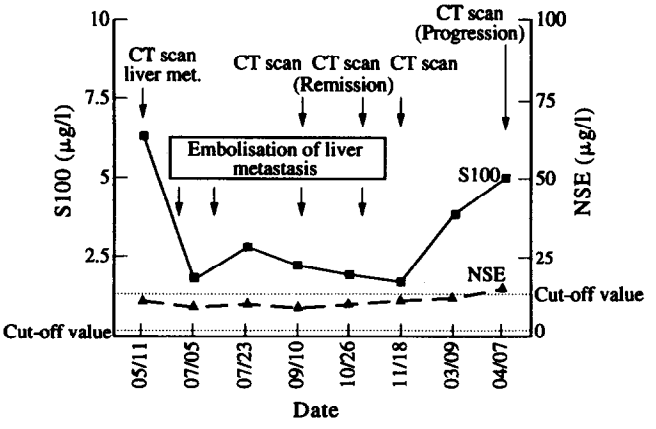


Figure 6. Serum S100 and neuron-specific enolase concentrations in a 65-year-old female patient. Melanoma of the choroid membrane, stage IV (liver metastases, met.).

concentrations were always below cut-off value. Only the last measurement revealed a slightly increased serum NSE level.

In a 33-year-old woman (Figure 7), in whom thoracic and abdominal CT showed no distant metastases, serum S100 concentrations were elevated and markedly increased, despite two courses of immunotherapy with α-interferon and interleukin-2, reflecting clinical progression of the disease. The patient underwent two courses of cytotoxic therapy (bleomycin, vincristine, lomustine, dacarbazine). After a slight decline, serum S100 increased again and multiple lung metastases were found by CT scan. Serum NSE concentrations always remained below the cut-off value.

In a 45-year-old woman with stage IV disease, the first measurement showed elevated serum S100 and NSE concentrations. Metastases in the right lower lobe of the lung and visceral metastases were demonstrated by CT scan. During immunotherapy with α-interferon and interleukin-2, stable disease was achieved, and serum S100 remained slightly elevated. Afterwards, S100 and NSE levels increased again, and a thoracic and abdominal CT scan indicated progression of lung and visceral metastases. Lung metastases were then found in the left and the right lobes. Despite two courses of cytostatic therapy and further immunotherapy, CT scan demonstrated progressive

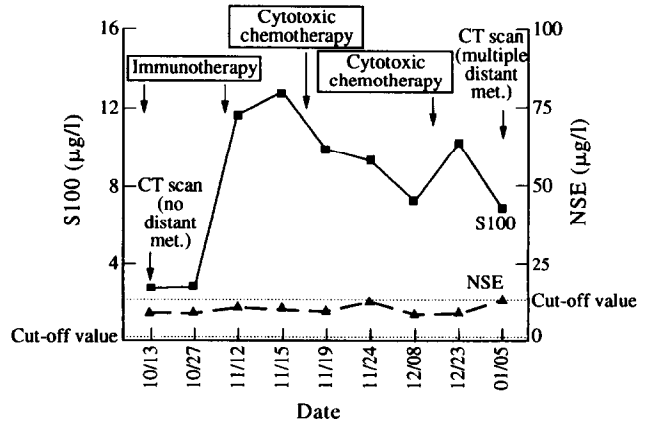


Figure 7. Serum S100 and neuron-specific enolase concentrations in a 33-year-old female patient. Blue naevus of the right medial thigh, stage III. met., metastasis.

lung metastases, and MRI scan showed intraspinal metastases in the level of the eight and ninth thoracic vertebra. The changes of serum NSE correlated with serum S100 throughout the whole course of the disease.

DISCUSSION

In 1980, it was reported that five of seven cell lines derived from human metastatic melanoma produce S100 protein [15]. Later it was demonstrated immunohistochemically that S100 protein is widely distributed among melanoma, especially of the amelanotic type, and is therefore a useful diagnostic indicator for malignant melanoma [6, 16]. Previous observations on the concentration of serum S100 suggested that serum S100 levels may be a useful marker of the stage of the disease [7].

The data presented here confirm serum S100 to have a interesting sensitivity (41.3%) for advanced disease (stage III/IV). 17 of 20 patients with initially detectable serum S100 had distant metastases, whereas elevated serum S100 concentrations were detected in only 2 patients with involvement of lymph nodes (stage III). In only 1 of 80 patients without metastases (stage I/II) did serum S100 exceed 0.15 μg/l. The low incidence of elevated serum S100 levels in patients without metastases indicates clearly that serum S100 is not useful for screening or for early diagnosis of the disease.

In the study presented here, serum S100 was serially determined in 6 patients with metastases (stage III/IV). To our knowledge, this study is the first to follow-up on the development of serum S100 in patients with metastatic melanoma. Serum S100 concentrations reflected the course of the disease during therapy. A persistent rise in serum S100 indicated progression of the disease, whereas a decline in serum S100 indicated response to therapy. Reflecting the fact that no complete remission was achieved, S100 remained detectable in all patients.

Previously, serum NSE was proposed as a prognostic factor in metastatic melanoma [4, 5]. In this study, the relationship between serum S100 and NSE in patients with malignant melanoma was investigated. In agreement with previously published data, in some patients serum NSE seemed to be a useful tumour marker for the monitoring of the efficacy of treatment and the progression of the disease. Nevertheless, its diagnostic sensitivity (23.9%) for patients with clinical stage III/IV versus patients with clinical stage I/II is lower than that of serum S100 (sensitivity 41.3%; $P < 0.05$). Serum S100 showed a better discrimination between patients with distant metastases (stage IV) and those without it. In each of the 6 patients in whom serum S100 and NSE were measured serially, serum S100 concentrations were above the cut-off value and reflected progression or remission of the disease, whereas in 2 of these patients NSE concentrations remained below the cut-off value. In the other 4 patients, serum S100 and NSE were correlated.

In conclusion, the current study supports the clinical significance of serum S100 in metastatic malignant melanoma. Serum S100 showed a higher sensitivity than serum NSE and correlated with the clinical stage of the tumour. Serial measurements of S100 were helpful in monitoring the treatment.

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